Bio-Speedy® SARS CoV-2 Triple Gene RT-qPCR Kit

Cat. No: BS-SY-WCOR-308-100
BS-SY-WCOR-308-250
BS-SY-WCOR-308-500
BS-SY-WCOR-308-1000

Package Insert

Manual Version: 201221-7
Approval Date for Use: 21.12.2020

For in vitro diagnostic use only.
For laboratory and professional use only.

Table 1. Kit content [**Shelf Life:** 18 months; refer to the expiration date on the box. Each reagent stored at storage temperature, may be used until the expiration date indicated on the tube. The expiration date of the kit is determined by the expiration date of the reagents].

Storage Temperature: -20°C, Transport Temperature: +2-8°C						
Content/Intended Use	**Content**	**Quantity (10 µL Reactions)**				**Consumption / Reaction**
		100 Rxns	**250 Rxns**	**500 Rxns**	**1000 Rxns**	
SARS-CoV-2 (RdRp), SARS-CoV-2 (N) and SARS-CoV-2 (ORF1ab) genes (**FAM**)	CVD Tri Oligo Mix	1 x 250 µL	1 x 625 µL	1 x 1250 µL	2 x 1250 µL	2.5 µL
Internal Control (IC) (RNase P) (**HEX**)						
DNA polymerase, dNTP mix, reaction buffer, reverse transcriptase and ribonuclease inhibitor	2X Prime Script Mix	1 x 500 µL	1 x 1250 µL	2 x 1250 µL	4 x 1250 µL	5 µL
Storage Temperature: +2-8°C/-20°C; Transport Temperature +2-8°C/-20°C If the components are frozen store at -20°C, it should be stored at 2-8 °C without being frozen again after thawing.						
Negative Control Template **Test it in each run for contamination control**	NTC	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL	2.5 µL
Positive Control Template: Synthetic SARS-CoV-2 and RnaseP genom fragment **Test it in each run for reactive stability control**	PC CVD Tri	1 x 250 µL	1 x 250 µL	1 x 500 µL	2 x 500 µL	2.5 µL

Instruments and equipments supplied by the user

1) Real-Time PCR Instrument: FAM/HEX/ROX/Cy5 (Green/Yellow/Orange/Red) channel, Ramp rate ≥3 °C/sec.
2) 1-10 µL, 10-100 µL and 100-1000 µL micropipettes and the compatible filtered tips (DNase and RNase free)
3) Quick Spin Centrifuge: min. 3000 rpm
4) Vortex
5) Nuclease-free water/Viral Transport Medium/Serum physiologic
6) 1.5 or 2 mL microcentrifuge tubes
7) Reaction tubes and their caps/seals compatible with the qPCR instrument and the reaction volume,
Extra components recommended to use:
8) UV Cabinet for PCR Setup
9) Cold Tube Rack (for microcentrifuge tubes and PCR tubes/strips)
10) Disposable powder-free nitrile gloves

Intended Use and Test Principle

Kit is used for detecting the epidemic virus SARS-CoV-2 causing Coronavirus Disease 2019 (COVID-19). ***The kit allows to achieve RT-qPCR result in less than 50 minutes***. The kit is applied to nucleic acid extracts obtained with vNAT extraction buffer or robotic extraction systems from *nasopharyngeal aspirate/lavage, bronchoalveolar lavage, nasopharyngeal swab, oropharyngeal swab and sputum samples*. Rapid diagnosis with the kit is achieved via one-step reverse transcription (RT) and real-time PCR (qPCR) (RT-qPCR) targeting SARS-CoV-2 specific *N* and *ORF1ab* genes fragment. The oligonucleotide set targeting human *RNase P* gene functions as a control of the sampling, nucleic acid extraction and inhibition.

Analytical Specifications

The kit is validated with RINA™ M14 Nucleic Acid Extraction System (Robot Cat No: RINA-M14-01; Consumables Cat No: RN-NA-14-111-100) and vNAT Extraction Consumables (vNAT Viral Nucleic Acid Buffer Cat No: BS-NA-510 ; vNAT Transfer Tube Cat No: BS-NA-513-100). The kit is validated for 10 and 20 µL qPCR volumes using Roche LightCycler® 96, Bio-Rad CFX96 TouchTM, Bio Molecular Systems Magnetic Induction Cycler (MIC), Qiagen Rotor-Gene® 5 Plex Real-Time PCR systems. The LOD of the kit is 5.6 SARS CoV-2 genome / reaction for all kit versions. The exclusivity tests of the kit were tested both in the laboratory and in-silico using a pool sample prepared with 40 different viral and bacterial strains and nasal washing fluids obtained from 20 different people. The kit does not cross-react with other respiratory pathogens and human respiratory microbial flora. In-silico tests have shown that the kit cross-reacts with some bat-associated SARS-CoV strains.

Sensitivity and specificity of the kit; Tested on 357 positive and 94 negative clinical specimens archived according to the FDA approved RT-qPCR assay kit targeting SARS-CoV-2. The relative sensitivity and specificity of the kit were determined to be 97.3% and 100% for all kit versions.

Collection, Storage and Shipment of Clinical Specimens

Swab samples should be collected using Dacron or Polyester swabs. Other specimen types should be transferred in sterile containers. In the transport phase, Viral Transport Medium (VTM) (Preperation of viral transport medium, Center for Disease Control and Prevention, SOP#: DSR-052-01) or *Bio-Speedy® vNAT Viral Transfer Tubes (Cat No:BS-NA-513-100).* Samples should be stored and transported at 2-8 °C until they arrive at the laboratory. Swab samples should be transferred within 5 days, other sample types should be transferred within 2 days. If a delay in shipment is expected, samples should be frozen at -70°C and shipped with dry ice. It is important that samples are not exposed to continuous freeze-thaw exposure.

Warnings

1. The kit should be stored away from nucleic acid sources and qPCR amplicons.
2. The components in the kit should not be mixed with components with different lot numbers or chemicals of the same name but from different manufacturers.
3. Master stock reagents should be kept on the cold block during the PCR setup; if possible, the PCR setup should be performed on the cold block.
4. Kit components should be mixed by gently shaking before use.
5. The micropipettes used for pipetting qPCR mixes and template nucleic acids should be separate.
6. Template nucleic acid and positive control tubes should always be kept closed, except for fluid transfers.
7. The wipeable surfaces of the rooms, benches and devices where the test is performed should be cleaned regularly with 10% NaClO.
8. The qPCR completed reaction tubes should be disposed of before opening in the laboratory.

RT-qPCR Application Protocol

Before starting the assay, please consider the following:

- The kit was validated only for the template nucleic acid volume that is 25% of the total qPCR volume.
- The kit can not be used with real-time PCR instruments without the periodic maintenance records.
- Both white and clear 0.1 mL qPCR plates can be used for the assay, while slightly better performance can be obtained using the white plates for Bio-Rad CFX 96 and Roche Light Cycler 96 instruments.
- 0.1 ml and 0.2 ml clear qPCR tubes can be used for the assay, while slightly better performance can be obtained using the 0.1 ml tubes for Rotor Gene Q instrument.
- Device-specific reaction strips are used in the BMS MIC qPCR device.

Program the qPCR device as follows and add the reagents to the qPCR tubes in the order specified below, close the tubes, place them into the qPCR device and start the run (Table 2).

Table 2. Reaction set-up and qPCR program details

Reaction setup			qPCR Program		
Component	**Reaction**		**Cycle Number**	**Temperature**	**Duration**
2X Prime Script Mix	5 µL	10 µL	1	52 °C	5 min
Oligo Mix	2.5 µL	5 µL	1	95 °C	10 sec
			40	95 °C	1 sec
Template Nucleic Acid	2.5 µL	5 µL		55 °C	1 sec
TOTAL REACTION VOLUME	10 µL	20 µL		FAM/HEX/ROX/Cy5 Read* (Green/Yellow/Orange/Red Read)	

Interpretation of The Assay Results

The recommended threshold levels to calculate the number of threshold cycles (Cq) for both 10 µL and 20 µL reactions are 0.05 and 200 RFU for Roche LightCycler® 96, Bio-Rad CFX96 Touch™ respectively. In Rotor-Gene® instruments, the sigmoidality of the amplification curves should be evaluated from the "Raw Data" screen. To see the Ct values of sigmoidal curves in Rotor-Gene® devices; on the analysis screen, "**Dynamic Tube**" should be active, "**Slope Correct**" options should be passive, "**Outlier Removal**" option should be "0", the threshold level should be set to 0.02. For BMS MIC qPCR, "**Non-Assay Green/Parameters/Fixed Length**" options should be selected, auto-threshold setting should be active. Shape of the amplification curves obtained in the FAM/HEX/ROX/Cy5 (Green / Yellow / Orange / Red) channels are examined and **non-sigmoidal curves are recorded as negative**. **The result is recorded as negative if there is no sigmoidal curve.** The result is recorded as positive if Cq<38 and the analysis result should be interpreted according to Table 3. **If there is no sigmoidal curve in the negative control and the sample is Cq≥38, the nucleic acid extract from the sample should be tested again, and if Cq≥38 is in the second analysis, a sample should be requested from this patient again and the analysis should be repeated.**

Table 3. Interpretation of Patient Samples

Cases	NA Isolate		Positive Control		Negative Control		Comment
	Target	**IC**	**Target**	**IC**	**Target**	**IC**	
Case 1	Pos	Pos	Pos	Pos	Neg	Neg	Target Positive
Case 2	Pos	Pos	Neg	Neg	Neg	Neg	Target Positive: Since the sample gives a positive result, it is concluded that the kit works, the patient result is reported.If the problem persists in the positive control, contact the manufacturer and request a new positive control.
Case 3	Pos	Neg	Pos	Pos	Neg	Neg	Target Positive
Case 4	Neg	Pos	Pos	Pos	Neg	Neg	Target Negative
Case 5	Pos	Pos	Pos	Pos	Pos	Neg	Contamination Problem: The experiment is repeated by paying attention to the issues in the warnings section.
Case 6	Neg	Pos	Pos	Pos	Pos	Neg	Target Negative: Since the target is negative, there is no contamination problem. NTC supplied with the kit contents may be contaminated. If the problem persists, the manufacturer is contacted and a new negative control is requested.
Case 7	Neg	Neg	Pos	Pos	Neg	Neg	Sampling / Extraction / Inhibition Problem: Nucleic acid isolate is diluted 1/10 and the experiment is repeated. If the diluted sample does not produce positive result in the HEX channel, a new sample is requested.
Case 8	Neg	Neg	Neg	Neg	Neg	Neg	Reagent Problem: Reagents are renewed by contacting the manufacturer and the reaction is repeated.

Case 9	Neg	Pos	Pos	Pos	Neg	Pos	If the IC Cq value in the negative control reaction is 3 cycles or greater than the IC Cq value obtained from the NA isolate, the result is evaluated and reported as negative. Otherwise, a sampling / extraction / inhibition problem is concluded.
Case 10	Pos	Pos	Pos	Pos	Neg	Pos	Target Positive: Since the target is negative in the negative c ontrol and the target is positive in the NA isolate, the sample is reported as positive regardless of the contamination in the IC.

Limitations

- Performance of the *Bio-Speedy® SARS CoV-2 Triple Gene RT-qPCR Kit* has only been established in nasopharyngeal swab, oropharyngeal (throat) swab, nasopharyngeal aspirate or lavage, bronchoalveolar lavage and sputum samples.
- Mutations within the target regions of the *Bio-Speedy® SARS CoV-2 Triple Gene RT-qPCR Kit* could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled.
- Inhibitors or other types of interference may produce a false negative result. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- Detection of SARS-CoV-2 RNA may be affected by patient factors (e.g., presence of symptoms), and/or stage of infection.
- Based on the in-silico analysis, other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the kit. Other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 are not known to be currently circulating in the human population, therefore are highly unlikely to be present in patient specimens.

WARNINGS: On the web page linked with the QR code, examples of sigmoidal and non-sigmoidal curves are given for different device types. The results obtained with this kit should NOT be interpreted without examining these samples.